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THE AMERICAN
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AUGUST, 1916

CITRIC ACID BY FERMENTATION.

By JOHN ALBERT MARTIN, P.D.

The replacement of natural products by artificially prepared substances is, with little doubt, one of the most interesting subjects to the scientist of to-day. This substitution has been the growing tendency within the last ten years, and nearly every one is aware of the wonderful advancements which have been made along these lines, especially in the science of chemistry—an example being in the commercial synthesis of gum camphor and the many synthetic products from coal-tar compounds, which have proved of such value and importance. Until recent years the artificial production of chemical substances has been accomplished either by the synthesis or analysis of other chemicals. But the investigations by some of our later scientists of fermentation and enzyme action have brought to light many of the heretofore unknown facts and the realization of the importance and possibilities of this action. As yet little commercial utilization of this knowledge has been attempted, although as far back as 1884, in Germany, it was found that citric acid, resembling the natural product in all its properties, could be formed, under certain conditions, by the fermentative action of certain microörganisms upon suitable carbohydrates. To our knowledge this was never made a commercial possibility in this country, and upon this consideration the following work is based. In selecting this subject for a thesis it is with the realization that it is one which would be considered by those of knowledge on fermentative action as quite an undertaking; however, with the facilities of the present time for obtaining the complete data and the literature in the many scientific journals on the recent results of experimentation in fermentation the results are encouraging.

The subject of citric acid production will be considered by first giving the present source of the acid, with a brief outline of its production and manufacture. Following this will be given its possible synthesis and its production by fermentation. The major part of this thesis consists of original work with the aid of Professor Kraemer, and takes in the period of experimentation from September, 1915, to April, 1916.

Citric acid is a well-known substance in many industries and sciences, especially in that of Pharmacy, in which it is one of the official acids in the United States Pharmacopœia and enters thirteen official preparations. It occurs naturally in the juices of many plants associated with other acids, especially in the fruits of the lemon, lime, bergamot, gooseberry, and in several bulbs and tubers. It was first obtained in the solid state by Karl Wilhelm Scheele in 1784 from the juice of the lemon. Citric acid occurs in colorless, translucent right rhombic prisms; odorless, having an agreeable purely acid taste, efflorescent in warm air and deliquescent when exposed to moist air. Citric acid was brought to the attention of the pharmacist in the early part of 1915 by the fluctuation in price, due mostly to the present European war and the destruction of the lemon groves by volcanoes and earthquakes which occurred recently in the southern part of Europe. This war, proving of such endurance and thereby affecting the transportation from all the foreign countries, has advanced the price of the acid over a hundred per cent.

Citrus Industry.

Citric acid is manufactured from the juice of the fruit of the following three species: *Citrus medica* (cultivated lemon), *Citrus Bergamia* (bergamot), and *Citrus Limetta* (wild lemon). The cultivated lemon is the main source and is grown most extensively in Sicily, Calabria, and, to a considerable extent, in Spain. Under favorable conditions a good lemon plant should yield one thousand lemons a year. The refuse lemons, which form about one-fourth the crop, are used in the manufacture of citric acid, as they cost only half as much as the picked fruit. A skilled operator can peel more than four thousand lemons a day, the peel being collected separately for the preparation of the volatile oil, which is an important branch of this industry.

The preparation of the juice for shipment is comparatively a simple matter. The presence of glucose with certain extractive gummy substances and inorganic salts in the fresh juice renders it impossible to crystallize the citric acid by concentrating the juice, even when all the glucose is transformed into alcohol. So, even at the present time, the citric acid is separated by Scheele's costly process, by which it is first converted into calcium citrate. The high price of fuel has prevented the establishment of the citric acid industry in Sicily, and the preparation of the acid has been monopolized for a long time by England, Germany, and the United States. These countries receive the raw material from Sicily in three forms: first, as lemons packed in barrels containing sea-water; second, as concentrated juice called "Argo Cotto," but mostly as calcium citrate. The juice does not keep well and is usually concentrated at once in open pans with direct heat until the product is a blackish decoction. The boiling-hot decoction is passed through a cloth and collected in casks for transportation.

Treatment of the Juice.—In the large modern factories, where the juice of the lemon is employed, the concentrated liquid is first clarified from its extractive matter by allowing it to ferment. It is then filtered and brought to the boiling temperature and neutralized with dense milk of lime or with powdered calcium carbonate. The cakes of calcium citrate thus formed are taken from the filter presses and mixed with water. The lime of the calcium citrate is neutralized with dilute sulphuric acid, liberating free citric acid and precipitating calcium sulphate.

Concentration was formerly carried out in lead-lined wooden vessels by rapid evaporation, and at 46° C. almost all the calcium sulphate is precipitated. There are three modern factories in this country which use the vacuum apparatus as in the sugar industry. The clear liquid is then siphoned into a vessel beneath and the concentration continued. After decolorizing the liquid by passing through charcoal, the liquid is finally transferred to wooden vessels and crystallization repeated until colorless, translucent crystals are obtained.

Citric Acid by Synthesis.—The acid has been prepared synthetically by Grimaux & Adam. A saturated solution of dichloracetonic acid was neutralized with sodium carbonate and heated with two molecules of potassium cyanide. The resulting solution of dicyanoacetates was saturated with hydrochloric acid gas and heated on a

water-bath for fifteen hours. The citric acid was then separated as calcium citrate by neutralization with milk of lime.

Another synthesis has been effected by Lawrence, who obtained ethyl citrate by heating together ethyl bromacetate and ethyl ozalyl acetate in the presence of zinc. Both, however, are impracticable.

In considering the subject of citric acid by fermentation it is important to mention the recent theories on the action of ferments and the cause of fermentation in order to understand from which view the acid formation was studied.

The first two prominent theories accounting for the cause of fermentation were: one, in which the action was regarded as a chemical change of the substance itself without the assistance of any organic body; the other, that fermentation was due to the action of an organism. The latter was proved to be correct and was studied thoroughly by a number of prominent scientists. It was found that for fermentation there must be present a ferment, organic matter, proper temperature, and water to allow free molecular motion. Noted bacteriologists and chemists such as Turpen held that the presence of the fungi caused a breaking down of the sugar to carbon dioxide and alcohol; while Liebig believed the transformation of the sugar was caused by the action of substances, later determined to be enzymes, contained in the fungus itself.

Pasteur, in 1812, showed that certain ferments live at the expense of the oxygen of the air and can decompose sugar into carbon dioxide and water, but when they are immersed in the saccharine fluids and no longer able to obtain oxygen from the air they would extract it from the sugar, changing it into carbon dioxide and alcohol. Liebig's theory, confirmed by the experiments of Pasteur, led up to the present theory,—that fermentation is caused by the action of substances (enzymes) which are produced by the changing (breaking down) of the cell substances in the growth of the plant, the true ferment in a broad sense being a by-product of the organism's growth.

The presence of enzymes was experimentally proved by Büchner in 1900, who succeeded in showing that some of the fermentations in the past which could only be induced by living organisms could also be effected by using the extract of these organisms obtained by forcing it under great pressure through special unglazed porcelain filters, the cells being previously ground with quartz sand to rupture

the cell wall. The enzyme thus extracted possesses the same action as the growing yeast cell.

A distinction must be made between the two classes of ferments, organized and unorganized. The ferment considered in this work is produced by one of the fungi (Hyphomycetes) of the organized group and resembles the common blue mould found so freely on decaying vegetable matter.

Citric Acid by Fermentation.

In the year 1892 Carl Wehmer, a Hanoverian botanist, while working with alcoholic fermentation, found citric acid in a fermenting sugar solution. After numerous experiments he proved this change was brought about by two separate species of moulds or filamentous fungi, named by him *Citromyces Pfefferianus* and *Citromyces glaber,* respectively.

These moulds were described as belonging to the Ascomycetes and resemble *Penicillium glaucum,* the blue mould which occurs so commonly on fruits and decaying proteids. The spores of the Citromyces are present in the atmosphere and the plant growth can be found on common fruits, such as the apple and pear.

Mucor piriformis, a filamentous fungi of the Zygomycetes group, was first discovered by Alfred Fischer and studied by Carl Wehmer, who showed that it forms comparatively large quantities of citric acid when light is admitted to cultures of the fungus grown in a suitable nutrient solution. The spores of this plant also occur in the atmosphere and were first found on decayed fruit.

From the above, it appears that three different species of fungi have been shown to produce citric acid, but that comparatively little work has been done by the authors to find the value of this formation, while up to the present time no attempt has been made in this country to find if this formation is of commercial value.

In the preparation of this work it was first necessary to secure the fungi described by the authors, and, as there had been no verified cultures of these species of fungi sent to this country, it was left to look for them as they occurred naturally. Pure cultures were obtained by following the usual method of sterilizing a piece of platinum wire by passing through a flame and, after becoming cool, touching the surface of the mould growth desired and, with the few spores adhering to the wire, inoculating the sterilized nutrient

substance prepared. The culture was then labelled and allowed to stand exposed to light and an average temperature of 20° C., taking note of its appearance at different stages of growth.

A culture was obtained from the U. S. Department of Agriculture which was said to resemble the Citromyces in its acid production. This specimen was labelled No. 28.

Through the assistance of Professor Kraemer two cultures of *Mucor piriformis* were secured from the International Botanical Association, Amsterdam, Holland.

Of the total number of specimens taken, twelve distinctly different genera were selected to determine their acid-forming properties, including the verified culture of *Mucor piriformis,* the remaining thirty-one specimens being identified as various species of *Penicillium, Aspergillus,* and *Mucor.* The fermentative action of these three genera being well known, they were discarded.

These common moulds are readily distinguished microscopically, although with the naked eye they occur in many distinct and characteristic colors.

Aspergillus has seven common species, all of distinctly different color. (It can be seen from this that a number of the same genus can appear as entirely different plants.)

Examination of Specimens.

The time required to detect the growth with the naked eye was from three to seven days. After inoculation of the cultures, each specimen was examined at the first appearance of growth and at in-

Table I.

Observations of Specimens Growth on Solid Cultures.

Specimen number	Identification	First growth, days	Color: Fifth day	Color: Ten days	Color: Twenty days	Reproduced by
25	Mucor piriformis	2	Black	Black	Brown	Sporangia.
24	Mucor mucedo	2	Black	Black	Black	Sporangia.
33	Mucor racemosus	3	Black	Black	Brown	Sporangia.
31	Mucor ? *	3	White	Brown	Brown	Sporangia.
28	Citromyces	4	White	Pale green	Light brown	Conidia.
14	Citromyces	3	White	Dark green	Dark brown	Conidia.
19	Penicillium, G.	2	Pale green	Dark green	Dark green	Conidia.
22	Penicillium ? *	2	Pale green	Dark green	Brown	Conidia.
30	Aspergillus N.	2	White	Black	Brown	Conidia.
21	Aspergillus A.	3	White	White	White	Conidia.
9	Aspergillus F.	4	White	Yellow	Brown	Conidia.
12	Aspergillus ?	3	White	Dark green	Dark green	Conidia.

* Identification of these fungi not determined.

tervals one or two days after. The notes on the following page were taken during two weeks' time. After having secured the pure cultures of these known species of fungi, the object was to find their fermentative power and production of acid, if any. To do this it was necessary to have a sterile liquid containing no harmful substances but those which would support the growth of the plant and a known quantity of sugar from which the acid may be formed.

The following nutritive solution was suggested by Professor Kraemer and used with success:

Ammonium phosphate, $(NH_4)_2HPO_3$	3.9	Gms.
Acid potassium phosphate, (K_2HPO_3)	1.05	Gms.
Magnesium sulphate, $MgSO_4$	.05	Gms.
Calcium nitrate, $Ca(NO_3)_2$	.05	Gms.
Dextrose	100	Cc.
Distilled water	1000	Cc.

Difficulty was had at first in the preparation of this solution, as the precipitate of magnesium ammonium phosphate was formed in the completed mixture, but this was overcome by dissolving the magnesium salt and the ammonium salt each in 500 Cc. of water. On then mixing these two dilute solutions no precipitate was formed, the remaining salts being finally added. One hundred cubic centimetres of this solution were placed in each one of twelve Erlenmeyer flasks and twelve disk-shape flasks, and the neck of each plugged lightly with cotton wool. These were then placed in a steam sterilizer and kept until perfect sterilization was obtained.

The now sterile liquid cultures were left standing at 20° C. for six days. The absence of living bacteria and fungi being assured, each was inoculated with one of the known specimens and labelled with number and date. The inoculating was done as in the preparation of the solid cultures, being careful to have the wire sterile and only the spores of the mould desired planted in the solution, as any contamination would make the attempt to find the acid-producing powers of the specimen useless. The cultures were allowed to stand at an average temperature of 20° C. exposed to light for ten days, it requiring that length of time for the surface of specimen No. 28 and No. 25 to become covered, the remaining requiring from six to eight days to reach their maximum growth. There being found no decisive change on examination of the solution after five days' standing, it was reasoned that on the average the growth reached its optimum

in ten days and maximum in twenty days; the condition of the solution at this time was proper for determining the acid content. On the tenth day the solutions were filtered and 10 Cc. of each set aside for immediate titration. The flasks were sterilized and the identical procedure was carried out for the second time, but allowing the growth to continue for twenty days.

These solutions were also filtered and 10 Cc. of each titrated. The results of this work are given in Tables II and III.

TABLE II.

Acid Content in Solution After Ten Days' Standing, Using Empirical (V. S.) NaOHn/20 as Titre. (Blank Test—10 Cc. Nutrient Solution Req. 4.2 Cc. NaOH (V. S.).

Culture number	Amount taken	Burette reading	Amount required	Excess required	Acid recognized
	Cc.				
9	10	0– 4.2	4.2	0	....
12	10	4.2– 8.4	4.2	0	....
14	10	8.4–15.3	6.9	2.7	as $H_3C_6H_5O_7$.
19	10	15.3–19.5	4.2	0	....
21	10	19.5–24.2	4.7	0.5	?
22	10	24.2–28.4	4.2	0	....
25	10	28.4–32.6	4.2	0	....
28	10	32.6–48.4	15.8	11.6	as $H_3C_6H_5O_7$.
24	10	0– 4.2	4.2	0	....
30	10	4.2– 9.8	5.6	1.4	as $H_2C_2O_4$.
31	10	9.8–14	4.2	0	....
33	10	14 –18.2	4.2	0	....

TABLE III.

Acid Content in Solution After Twenty Days. Empirical V. S. NaOHn/20 as Titre Ph. Indic. Blank Test—10 Cc. Nutrient Solution Req. 4.2 Cc. NaOH (V. S.).

Culture number	Amount taken	Burette reading	Amount required	Excess required	Acid recognized
	Cc.				
9	10	0– 4.2	4.2	0	....
12	10	4.2– 8.4	4.2	0	....
14	10	8.4–18.3	9.9	5.7	as $H_3C_6H_5O_7$.
19	10	18.3–22.5	4.2	0	....
21	10	22.5–27.2	4.7	0.5	?
22	10	27.2–31.6	4.2	0	....
25	10	31.6–35.8	4.2	0	....
28	10	0–47.1	47.1	42.9	as $H_3C_6H_5O_7$.
24	10	0– 4.2	4.2	0	....
30	10	4.2–22.7	18.5	14.3	as $H_2C_2O_4$.
31	10	0– 4.2	4.2	0	....
33	10	4.2– 8.4	4.2	0	....

The result of this titration was to find only the quantity of acids formed corresponding to a known strength alkaline solution, as the presence of different complex acids through the action of the fungous growth and the nutrient salts which would be in the solution could not all be estimated as one definite acid. The fifth column of the table shows decided results, there being only four specimens which had formed acid. This eliminated all the remaining specimens, the work now being reduced to these four: Nos. 28, 14, 30, and 25.

The other specimens, being of no further interest as to acid production, were dropped. Identical results were found with the solutions in the flat-bottom flasks, showing that the excess of air in this case was of no consequence.

Three tests were used to detect the presence of citric acid, one by the use of calcium citrate, and two by color reactions which gave decided confirmative tests, both of these being given under "Chemistry" portion of this thesis.

Of the four specimens taken, Nos. 28 and 14 responded to the above test, both giving distinct reactions. This brings the number down to two, eliminating No. 25, *Mucor piriformis,* which was mentioned by La Far as having produced citric acid. The failure of this specimen to respond was probably due to unfavorable conditions of temperature and light (which were studied in detail to determine the plant's activity, but without results). No. 30, *Aspergillus nigrescens,* showed a strong reaction to oxalic acid tests. Repeated experiments were made with specimens Nos. 28 and 14 to determine any additional acid-forming properties, and in each case No. 28 was found to be far the more active, yielding nearly eighty per cent. more acid, although being of slower growth (and easily overgrown by No. 14). The whole remaining work and experiments were concentrated on this one species of Citromyces, studying its action under different conditions of temperature, in various culture flasks, and mainly its action on different nutrient solutions. It was found to be independent of light. The final object was to determine the maximum quantity of crystallized citric acid which could be produced by this plant, taking careful observation of the conditions required for this result. The above work is given under "Acid Production" by Specimen No. 28.

Microscopical Examination of Specimens.

A detailed description of all the specimens used would be of little value, therefore the study of only the specimens producing acid are to be considered.

No. 28, secured from the United States Department of Agriculture, was found to closely resemble the Citromyces group, and was first examined macroscopically, with the following notes: The appearance of the plant on a solid culture the fourth day after inoculation was a white wool-like growth of spherical shape (with convex surface gradually spreading over the surface equally in all directions). The fifth day a pale-green color appeared at the centre, due to the production of spores, which rapidly covered the surface, this color remaining characteristic throughout the plant's growth, the maximum growth being from a week to fifteen days, according to the temperature, the optimum 25° C. The plant at this stage gradually dies, turning brown, but the spores retain their activity and readily germinate on fresh nutrient media. Gelatin media was liquefied after six days' growth and was found less satisfactory than agar media, which not only remained solid but was less prone to drying. The time for minimum and maximum growth is found in Table I. In liquid cultures, growth was much slower, the minimum time required for growth to appear being six days, and from twenty to thirty days for maximum growth, according to temperature.

In a liquid culture where air was excluded, there was no color produced, the growth remaining white and of uneven convex surface concentrated in spots. This shows that an excess of air is necessary for spore production, and this species is probably what Wehmer named *Citromyces tollensianus,* given in the *Journal of Society of Chemical Industry,* 1910, p. 37.

On allowing air to pass through cotton wool into the flask spores were produced after the second day. Under the microscope a slide made from the surface growth on solid culture showed a mycelium consisting of transparent, branched, and divided filaments which usually swell somewhat irregularly. From these filaments the conidiophores rise perpendicularly. They consist of an elongated cylinder, the end cell of which is soon arrested in its growth and forms a thorn-like summit (the cell immediately below throws out four or five opposite condiospores, rising up close to the end cell, forming a fan-shaped organ).

At the summit of each of these branches is an elongation in the plant's growth by which the upper extremity is constricted from the main plant, forming a new independent organ or spores. The spore formation, which is continuous throughout the growth of the plants, shows a chain of spores remaining attached to each other by a

remaining thread, which is easily broken by the force of a draft of air or in coming in contact with some moving object.

In liquid media the mycelium ramifies in all directions, producing pairs of thick, short branches which entwine themselves about each other, forming in groups of loose masses with the formation of perithicea, an organ of reproduction which consists in this case of a spherical interwoven mass of mycelia threads having the property of producing asci. These organs are very resistant to sterilization, due to the protective coat of mycelia.

Upon examination, No. 14 proved to be similar to No. 28. The method of reproduction of the plant was exactly the same, while the growth was nearly twice as rapid. The formation of perithicea also occurred in liquid cultures, but not to such an extent as with specimen No. 28. The surface color of the plant is a much darker shade of green and easily distinguished, the study of the conidiophore showing a larger and more heavily branched formation and, consequently, more spores, which accounts for its more rapid growth.

The presence of such large numbers of spores made it very difficult to examine the structure of the conidiophore, and required the examination of the specimen to be made at an early stage of the plant's growth.

No. 25 (*Mucor piriformis*), although not giving results in its production of acid, was of very great interest, it being easily distinguished on account of its size and its clear method of reproduction by both sporangia and perithicea. The macroscopical appearance of the plant at the beginning of its growth is a white woolly growth from two to three millimetres high, spreading over the surface of the media gradually. After two days the growth, now from eight to ten millimetres in height, turns black on the surface, due to the formation of spore-bearing sporangia. The plant then rapidly covers the surface of the medium through the spreading of the spores. The first formation of the mycelia in liquid culture is followed by perithicea, which later, as in solid culture, produces a conidiophore (upright stem) bearing, as described, a sporangium or head, covering a pear-shaped columella between which the spores are contained. As the growth proceeds, the columella enlarges, absorbing moisture, and ramifies upwards, breaking the film covering the head and freeing the spores. This reproduction takes from twelve to fifteen days, and after this time the plant dies, turning to a brown color.

No. 31 (*Aspergillus nigrescens*) also proved of interest in its manner of reproduction, but was more like Citromyces in its spore-bearing organ. A short description is given of this plant, owing to its production of oxalic acid. The conidiophore supports numerous rod-shaped branches, each terminating in a spherical spore. *Aspergillus nigrescens* can be mistaken macroscopically for *Mucor* by its first appearance as a white woolly growth, later turning black, due to the spore formation.

No. 19 (*Penicillium glaucum*) was studied on account of its free occurrence and its readiness to contaminate pure cultures. It was found to be one of the most difficult to eliminate in the preparation of cultures, and would probably be the hardest thing to avoid in citric acid fermentation on a large scale, due to the sturdy character of the plant and its ability to outgrow any other fungus present. Macroscopically it is easily distinguished from specimen No. 28 by the marked difference in color of the two organisms, *Penicillium* being a dark green to a blue, while No. 28 is of a distinct pale green. It was quite difficult, however, to distinguish it in color from No. 14, which is but slightly lighter green than *Penicillium*. Microscopically the plants were comparatively easily recognized, *Penicillium* being much larger and more heavily branched. The reproduction is identical.

In the study of these fungi they were also grown upon Rawlin's nutritive solution. This was used chiefly in the propagation of cultures.

Rawlin's Nutritive Solution.

	Gm.
Distilled water	1500.00
Cane sugar	70.00
Tartaric acid	4.00
Ammonium nitrate	4.00
Ammonium phosphate	.60
Potassium carbonate	.60
Magnesium carbonate	.40
Ammonium sulphate	.25
Zinc sulphate	.07
Ferrous sulphate	.07
Potassium sulphate	.07

In the preparation of this solution, on adding the tartaric acid a precipitate is formed of ammonium magnesium phosphate, which, on substituting with citric acid, forms the double soluble salt of ammonium magnesium citrate, giving a clear solution. Two per cent.

of agar-agar is added and allowed to boil until all the agar is dissolved. Agar was found preferable to gelatin for making solid media, as the surface of the gelatin culture after exposure to air for a time would become too hard to allow the growth of the plant. The sugar is not added until this point, for if it is kept in contact with the heated acid too long it is caramelized, giving the solution a dark-brown color. The completed mixture was then placed in six-inch test-tubes about one-fourth full and carefully plugged with non-absorbent cotton, allowing the free passage of air, but obstructing the entrance of any contaminating substance. The cultures are sterilized at 100° C. for thirty minutes. This was repeated for three consecutive days and was finally free of all bacteria or plant life and ready for the reception of the mould spores.

Twelve other formulas were prepared, increasing or decreasing the amount of each ingredient, with the following results: The growth of the plant was not dependent on the presence of the nutrient salts, as the growth would proceed on the sugar alone, however, very slowly, it taking about twice the time without the nutrient salts as with them. With the omission of a portion of the citric acid the growth was comparatively slow to start, but would increase rapidly after germination began. The omission of the ammonium salts had a decided effect of retarding the growth, showing both the nitrogen and phosphorus were essential for the development of the fungus, as was potassium, which was shown very distinctly when the fungus was burned on a platinum wire, showing the violet potassium flame. The magnesium also showed a slight effect when its quantity was reduced. With the zinc no action was observed. It was found that without the presence of a minute quantity of iron the production of spores was not as plentiful.

On increasing the stated amount of any of these ingredients to a large degree the growth was hindered by the too rapid formation of acid or by-products, the effect of zinc sulphate in large amounts acting as a positive antiseptic, while the sugar, if over 15 per cent., prevents rapid growth.

The nutrient liquid cultures used for determining the acid production are stated in a former and following section.

Acid Production by Specimen No. 28.

The work leading up to the present division has been to isolate the most active species of fungi in the production of citric acid and

to determine the most advantageous conditions for the propagation of this plant. This having been determined, there will now be given the consideration of the plant's activity in its maximum production of acid under these conditions. As specimen No. 28, obtained from the United States Department of Agriculture, Bureau of Plant Industry, was found to be the one answering to these requirements, as was stated in a former section, this plant was used in the following work. Three flasks, each containing 100 Cc. of the 10 per cent. dextrose solution, were inoculated with the spores from the growth of specimen No. 28 on Rawlin's solid media, the fourth being reserved for control. The solutions were agitated gently to evenly distribute the spores and allowed to stand in the light at an average temperature of 20° C. It being a warm time of the year, this temperature was quite easily maintained. The first appearance of growth was on the fourth day. On the sixth day the solution was gently agitated to intimately mix the liquids, whose specific gravity might have been changed by action of the plant's growth, however, not shaken enough to disintegrate the layer of fungus growth on the surface. About 10 Cc. of the solution was taken from each culture by tilting the flask and allowing the liquid to flow out the side tube, thereby avoiding contamination from opening the mouth of the flask.

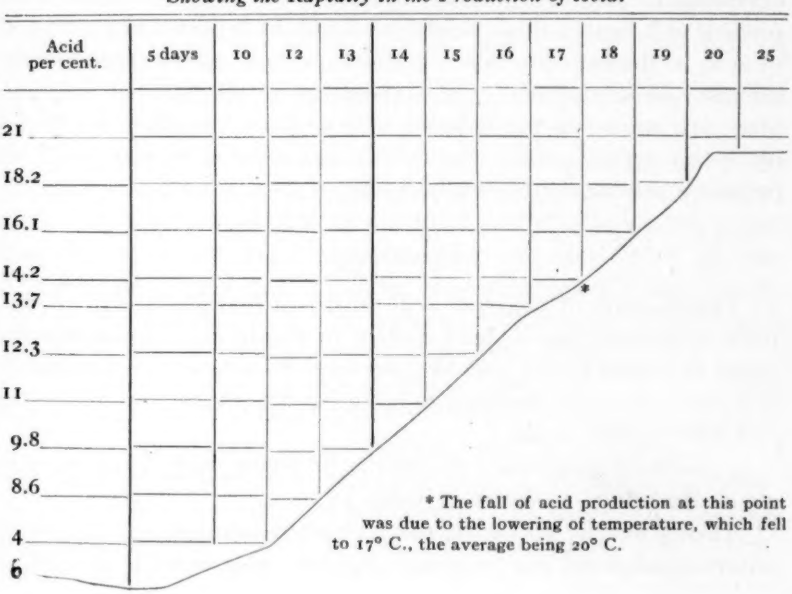
Exactly 10 Cc. of each of these solutions were titrated with NaOHV.S.n/10 after first being treated as given under section of "Chemistry." No acid was produced at this time, the amount of V. S. required for neutralization being equal to that of the blank test. On the twelfth, twenty-fourth, and thirtieth days the same procedure was carried out with the decided results as given in Table III. It was found, as shown on the chart, that after the twenty-fourth day there was little production of acid.

To carry out a more detailed examination of this acid production a fermentation jar was constructed along lines proposed by Professor Kraemer, in which a large quantity (5000 Cc.) of the nutrient solution could be acted upon. This jar consists of a three-gallon glass jar, covered at the top with wire netting, over which was placed non-absorbent cotton. To the wire netting was suspended a thermometer, a glass inlet tube, and a wire holding an evaporating dish 50 mm. above the surface of the liquid and containing KOH, its object being to absorb CO_2 given off from the plant's growth and to keep a circulation of air throughout the jar, which was found for this

purpose quite satisfactory. At the base of the jar was an outlet spigot for the withdrawal of the solution. The jar was sterilized by rinsing thoroughly with formaldehyde solution, then sealing with the cotton and allowing to stand ten days, the evaporation being complete in that time. The nutrient sugar solution was then run in and inoculated with a culture of No. 28. The growth was equally rapid as that in the small flask, but it was found in a somewhat illustrative way that the acid produced by the plant's growth remained only in a distinct brown layer of about 30 mm. depth at the surface of the liquid. This was proved to be the case by inserting a sterile pipette into the layer and withdrawing 10 Cc. for titration. This made the withdrawal of the liquid from the spigot at the bottom useless and resulted in the use of an enamel-ware pan, which gives the maximum amount of surface for the liquid used. One thousand cubic centimetres of a culture of the same formula were prepared and allowed to ferment in the shallow pan. Ten cubic centimetres of the solution were withdrawn from the centre of the acid layer with a sterile pipette at regular intervals. The results are given on the following chart. So far the determination of the amount of acid produced has

Table IV.

Showing the Rapidity in the Production of Acid.



* The fall of acid production at this point was due to the lowering of temperature, which fell to 17° C., the average being 20° C.

been only up to where the acid reaches a concentration of about 20 per cent., and at this point stopping any further production of acid.

To allow the fermentation to proceed there was added calcium carbonate, as was suggested by Wehmer and as is done in lactic acid fermentation. This neutralizes the excess of acid-forming, insoluble calcium citrate and liberates CO_2. One thousand cubic centimetres of solution No. 1, with 5 per cent. of calcium carbonate, were placed in the culture pan and sterilized by boiling; also three cultures of 100 Cc. each, with the same percentage of calcium carbonate. It was found that during the sterilization the nutrient salts were precipitated as insoluble carbonates, leaving the solution alkaline and impossible for the plant's growth. The preparation of the culture was repeated, omitting the calcium carbonate, which was added gradually as the fermentation proceeded, only enough at a time to partly neutralize the excess of acid, having previously sterilized the calcium carbonate to prevent contamination. In this way the fermentation could proceed as long as the dextrose and nutrient solution remained, but as an excess of 10 per cent. of the dextrose was found to be deleterious to the plant's growth, this percentage was maintained. By this method after sixty days the solution was analyzed as given in the section on "Chemistry" and found to contain 43 per cent. of crystallized citric acid. If the fermentation had been allowed to proceed still further there would, no doubt, have been a greater yield of acid, as theoretically eleven grammes of dextrose are equal to six grammes of citric acid. On evaporating this fermented solution, after first removing the nutrient salts as later described, the liquid reached a syrupy consistence, which was allowed to stand and on becoming cool deposited small crystals of citric acid.

Chemistry.

This division of the work will consist of first giving the calculations in determining the acid content of the fermented solutions as stated in Tables II, III, and IV, and then the different chemical tests and color reactions employed in detecting the presence of citric acid and other organic acids.

In examining specimen No. 28 the following method was used to obtain the results as shown in Table IV:

Taking exactly 10 Cc. of the fermented solution and 10 Cc. of unfermented solution as a blank test, there was added a solution of

lead acetate until neutral and no further precipitation. This threw down the insoluble lead citrate with lead phosphate and sulphate, the latter two being present as nutrient salts in the solution. This was filtered and washed thoroughly with dilute alcohol. The precipitate was suspended in water and H_2S gas passed through until all the lead citrate was changed to insoluble sulphide, liberating the citric acid. There were also phosphoric and sulphuric acids formed from their salts, but these were taken into consideration with the blank test in the unfermented solution.

The sulphide is separated by filtration and the filtrate boiled until no odor of H_2S is evolved. This solution is then titrated with NaOHV.S.n/10 Ph. indicator and calculated for percentage of citric acid, less the amount required for the blank solution as in former calculations.

In determining the percentage of citric acid as calcium citrate when calcium carbonate is used to neutralize the fermenting solution the liquid is first boiled with the calcium carbonate to throw down all the citric acid in solution, which also precipitates the nutrient salts. This is filtered while hot and washed with dilute alcohol. To the washed precipitate is added dilute sulphuric acid until exactly neutral; this is boiled and the calcium sulphate separated by filtration, leaving citric acid and phosphoric acid in solution, which is titrated with V.S.NaOHn/10, the phosphoric acid being taken care of by a blank test of the nutrient solution.

In determining the presence of acids in the fermented solutions quaiitatively the following tests were used:

The formation of calcium citrate by taking about 10 Cc. of fermented solution, add NH_4OH to neutralization and boil off excess of NH_3, allow to cool, and add solution of $CaCl_2$ to excess, which precipitates out the nutrient salts; filter and boil filtrate to near dryness, add solution of NH_4Cl, and repeat evaporation three times, and if the precipitate formed remains insoluble on diluting with NH_4Cl solution it indicates insoluble calcium citrate.

This precipitate of calcium citrate is verified by applying a color test as given by Haeusler (*Chem. Zeitung*), which is as follows: When to citric acid an aqueous-alcoholic solution of vanillin is added and the mixture is evaporated to dryness, the residue, when heated on a water-bath for 10 to 15 minutes with a few drops of 25 per cent. sulphuric acid, will be colored violet. On addition of water a green-colored solution is obtained which, by ammonia water, is turned deep

red. On acidulating the liquid is colored green, but the red color is restored when the liquid is made alkaline again with NH_4OH.

Haeusler found that tartaric, malic, oxalic, malonic acids, etc., do not give this reaction. A small quantity of the calcium citrate precipitate was taken and mixed with ten drops of the vanillin solution; this was allowed to stand a few minutes and three drops of 25 per cent. sulphuric acid were added, which liberated the free acid. Heat was gently applied, and when near dryness a very distinct and characteristic violet color appeared. On adding water an immediate green was produced, which changed to a deep red with ammonia water.

Blank color test—10 drops of 50 per cent. alcoholic solution of vanillin was heated with 13 drops of 25 per cent. sulphuric acid. On nearing dryness a yellow, then a brown, and then a violet color was produced. After cooling and adding water the brown color returned, intensified with ammonia.

Denige's reaction for the detection of citric acid in the presence of tartaric, oxalic, malic, sulphuric, and phosphoric acids is as follows:

Small quantities of citric acid are detected by heating the solution to boiling with one-twentieth of its volume of Denige's reagent (5 grammes mercuric oxide, 80 Cc. of water, 20 Cc. concentrated sulphuric acid), 3 to 10 drops of an approximately decinormal potassium permanganate solution being added, a white crystalline precipitate is formed at once.

The oxalic acid was determined by the formation of insoluble ammonium oxalate in a solution of acetic acid, this being obtained by first treating with calcium chloride, dissolving in boiling dilute sulphuric acid, and subsequent treatment with ammonia water and acetic acid.

Conclusions.

In summarizing this work, avoiding any detail which may have been confusing, the following conclusions have been reached.

Certain fungi, when allowed to grow on a solution of sugar, under proper conditions, will produce citric acid identical in properties to that which occurs in Nature.

The fungi for this action are of the Ascomycetes group and appear as specimen No. 28, which is probably a species of Citromyces as described by Wehmer and grows rapidly in a thin, velvety, green layer over the surface of the liquid.

Different species of this group of fungi have different powers of activity.

The conditions for this fermentation are:

A neutral or slightly acid solution containing proper nutrient salts and dextrose, not exceeding 10 per cent. (However, not dependent on this form of sugar alone.) The temperature should be carefully regulated.

The rapidity in the yield of acid is in proportion to its growth, which is dependent on the temperature, but appears to be independent of light.

It is necessary to have an excess of air for the conversion of the sugar into acid, as is seen by $C_6H_{12}O_6 + 3O = C_6H_8O_7 + 2H_2O$.

Therefore the flask for fermentation should be spacious and shallow, as the acid forms and remains in a layer at the surface of the liquid surrounding the mycelia of the plant.

The production of acid is a gradual rise until it reaches about 20 per cent. of the sugar used. At this concentration the growth of the plant is stopped.

A greater yield of acid may be had by neutralizing the acid formed with calcium carbonate.

This yield may be brought up to 50 per cent. of the sugar used, as may be shown theoretically by the ratio of $C_6H_{12}O_6 : C_6H_8O_7$.

From these statements it appears that this formation of citric acid by fermentation would be of value as a commercial consideration.

It would no doubt give rise to many difficulties at first and require further study and understanding of the action of the plant in this formation before an attempt could be made to produce the acid uniformly on a commercial scale.

This remains to be accomplished, and if properly considered should result in a satisfactory product.

THE PERCENTAGE OF ALCOHOL IN HOME-MADE ROOT BEER.[1]

By Charles H. LaWall.

It is a fact well known to chemists and biologists, as well as to many others, who by experience or training have been brought into contact with certain industries or have studied the subject theo-

[1] Read at the annual meeting of the American Pharmaceutical Association, June, 1916.

retically, that when yeast is added to any sugar-containing material and subjected to favorable conditions of temperature and moisture it immediately begins to grow and develop carbon dioxide and alcohol.

The carbon dioxide escapes as a gas and produces the phenomenon of effervescence, which gives the name " fermentation " to the operation, from the Latin *fervere,* to boil. The alcohol simultaneously produced sometimes remains in the finished product and sometimes escapes later on, by reason of its great volatility.

Even in bread making, where yeast is used, alcohol is present to an appreciable extent in the earlier stages of manufacture, and from 0.2 to 0.4 per cent. has been detected in a freshly baked loaf of bread, although the alcohol begins to escape as soon as the loaf is cut, and it is doubtful whether even the most minute traces could be detected in the ordinary bread of commerce.

The unfermented grape juice of the market always contains small amounts of alcohol, ranging from 0.05 per cent. up to 0.5 per cent., the higher amount being found in the carelessly prepared article.

It is very difficult indeed to get away from alcohol entirely. A rotting apple or other juicy fruit is likely to contain minute amounts; vinegar sometimes contains several per cent.; preserves or canned fruits which have started to " work " and have been resterilized contain it, and there are numerous other products which unavoidably and necessarily contain it.

In making some home-brewed root beer recently I suspected, from the physiological effect upon a person who drank a glass of it and who is very susceptible to alcohol, that more alcohol was present than is commonly supposed. The conditions under which the beverage is made are very favorable for the development of appreciable amounts of alcohol. Yeast, sugar, water, and a flavoring extract which usually contains some inorganic salts for the stimulation and nutrition of the yeast, are combined under conditions favorable to the rapid growth of the yeast, and the mixture is then bottled and the bottles are directed to be tightly closed.

When the pressure of carbon dioxide, evolved by the fermenting mixture, reaches a certain point, the fermentation automatically ceases. It may easily be seen that if the mixture is allowed to stand for a short time before bottling, or if the bottles are not entirely filled so that a comparatively large air space remains, fermentation may proceed for some time, and the alcohol content is correspondingly varied or increased.

I accordingly made some experiments to ascertain just how high the alcohol would go under the most favorable conditions, and also to see what the average alcohol content of a product made strictly according to directions would be. The following results were obtained:

After standing 2 days.....0.25 per cent. alcohol.
After standing 3 days.....0.32 per cent. alcohol.
After standing 4 days.....0.35 per cent. alcohol.
After standing 5 days.....0.53 per cent. alcohol.
After standing 6 days.....0.64 per cent. alcohol.
After standing 7 days.....0.81 per cent. alcohol.
After standing 9 days.....1.29 per cent. alcohol.
After standing 10 days.....1.36 per cent. alcohol.
After standing 11 days.....1.52 per cent. alcohol.

No higher alcohol content was observed in this series even after standing for ten days longer.

Later some additional experiments were made, allowing the fermenting liquid to stand for three hours before bottling and also only partially filling the bottles, and while, of course, the alcohol content rose more rapidly in each case, the highest amount noted under the most favorable circumstances was 1.77 per cent.

There is a very delicate question involved as to whether a beverage made in this manner could be sold as a non-alcoholic drink, using the expression "non-alcoholic" in its popular significance as equivalent to non-intoxicating. Under the internal revenue rulings we find No. 804, June 29, 1904, J. W. Yerkes, Commissioner, the following:

"The question whether fermented malt liquor is intoxicating or non-intoxicating is immaterial under the internal revenue laws, although it may be a material question under the prohibitory laws of a State or under local ordinances."

No ruling, so far as I can find, has ever been made with reference to root beer, nor can I find any literature on its alcohol content when made as above described. The soda-fountain root beer, of course, is made by diluting a flavored syrup with carbonated water and therefore contains no more alcohol than the minute amount contributed by the extract used to flavor the syrup, which would not exceed 0.05 per cent., and is not to be confused with the home-brewed or fermented product, which is the subject of this article.

It is recorded in literature that koumiss, which is made from milk fermented under somewhat similar conditions, sometimes contains over 2 per cent. of alcohol.

The foregoing facts may come as a surprise to many who have looked upon home-brewed root beer as a strictly temperance drink. With beer averaging 4 per cent. alcohol, the mathematical ratio becomes apparent that three bottles of home-brewed root beer, which have been allowed to stand for ten days or over, are equivalent to one bottle of ordinary beer.

SOME OF THE CHANGES MADE IN THE NINTH DECENNIAL REVISION OF THE UNITED STATES PHARMACOPŒIA.[1]

By George M. Beringer, A.M., Ph.M.

On the eve of the appearance of the Ninth Decennial Revision of the United States Pharmacopœia, it is very appropriate that the changes made in this legal authority for drugs should be discussed by pharmacists.

The present revisions of the Pharmacopœia and of the National Formulary are the first editions of these books to appear since they were specifically named in the Food and Drugs Act as the standards for the identity and quality of drugs. To revise the Pharmacopœia so that its standards shall properly fulfil this added responsibility has been the paramount thought of the revisers. Hence it may be observed that the Ninth Revision will be noteworthy for this purpose and its consequent aim at scientific accuracy, and this purpose has been the primary cause for many of the changes that will appear in this revision.

From a therapeutic standpoint, the changes made in the strength of the galenical preparations are, as a rule, negligible. For the most part, they have been minor and not sufficient to affect either the action or dosage of the preparations. As most of the potent remedies were brought into harmony with the Brussels International Protocol by the Eighth Decennial Revision, radical changes, such as were then made in the strengths of tincture of aconite and tincture of veratrum, are not now necessary.

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[1] Read at the meeting of the New Jersey Pharmaceutical Association, Long Branch, N. J., June 22, 1916.

It was to be expected that the advances and the changes occurring in medical practice, and likewise the progress of our knowledge of the composition of drugs and their therapeutic actions, would demonstrate that the requirements of that protocol must be revised. Absolute compliance with all of its provisions is already no longer possible. In the two revisions of the Pharmacopœia that have been made since the promulgation of the international protocol, we have given ample evidence of our good faith and adherence to the principles to which our nation as a participant in the Brussels Conference and as a signator to the protocol committed us.

A few of the variations between the requirements of the protocol and those of the United States Pharmacopœia are cited as examples, and the reasons for the non-acceptance of the international requirements are given. The protocol requires that aconite shall be "the tubers of the current year." In America this has never been considered practicable, as *Aconitum Napellus* is not indigenous nor cultivated in this country, and, under the conditions existing during the past two years, it would have been absolutely impossible to have complied with this requirement. Moreover, it is an established fact that aconite, properly stored, retains its activity for a long period, and hence this requirement was considered as unnecessary and has not been adopted in our Pharmacopœia. The protocol specifies that belladonna is to be "only the dried leaf." The commercial article is the dried leaves and tops, and hence the U. S. P. very correctly so defines it. The protocol contains no standards for the tincture or extract of belladonna, except that the latter shall contain "about 10 per cent. of moisture." The U. S. P. provides alkaloidal standards for both. The same criticism applies to the absence of standards in the protocol for the preparations of colchicum and hyoscyamus. The protocol directs that tincture of strophanthus shall be made from the seed and not de-fatted and with a menstruum of 70 per cent. alcohol. We know that the fat in strophanthus is exceedingly disagreeable and nauseating, and that this fat can be removed with purified petroleum benzin without the loss of strophanthin. Moreover, 70 per cent. alcohol will not extract the drug, and hence the U. S. P. improves on the international preparation by the preliminary de-fatting of the seed and the use of alcohol as the menstruum.

The use for which a preparation is commonly administered may, likewise, necessitate a deviation from the protocol, as adherence to

its provisions would possibly prove a menace to life. As examples: Bitter almond water is commonly administered in the United States as a vehicle or flavoring medium in fairly large doses, and hence it is directed by the U. S. P. to contain not more than a mere trace of HCN. The protocol requirement is 0.1 per cent. of HCN, and to follow this in the dosage in which bitter almond water is, at times, directed as a vehicle in the United States would be dangerous. The U. S. P. syrup of ipecac is seven times stronger than that of the protocol. As this preparation is commonly used in the United States as an emetic, it was not deemed advisable to so reduce the strength as to render it valueless as an emetic in croup and similar affections in which it is so commonly employed.

As examples of the more important changes of strength of galenicals may be mentioned unguentum hydrargyri dilutum from 33 per cent. to 30 per cent. of mercury in order to comply with the protocol, and syrupus acidi hydriodici, changed from 1.19 Gm. HI in 100 Cc. to from 1.3 Gm. to 1.45 Gm. in 100 mils.

The changes that have been made in the strength of the chemical products are, for the most part, such as were required by the commercial conditions and the quality of the products commonly dispensed as medicines. The principle of allowing for the proper variability of chemicals and for the natural variation in crude drugs has led to many modifications of the rubric requirements by which, instead of the fixed purity statements of the previous revision, there now appears in most of the monographs a variability allowance in accordance with determined conditions, and the limitations of such variability are officially defined. The following examples among the chemicals illustrate the desirability and practicability of this change, which, in many cases, has been only a rounding off of the requirements: The Eighth Revision required that diluted hydriodic acid should contain not less than 10 per cent. HI; the Ninth Revision will state from 9.5 to 10.5 per cent. HI. In the Eighth Revision, hydrochloric acid was required to contain not less than 31.9 per cent. HCl; the Ninth Revision will state from 32 to 33 per cent. HCl. In the Eighth Revision, ether was about 96 per cent. ethyl oxide; in the Ninth Revision it will be from 95.5 to 97.7 per cent.

The purity of a few official chemicals has been increased. As examples, the bromides of ammonium, potassium, and sodium have been each increased from 97 per cent. in the Eighth Revision to 98.5 per cent. of the respective absolute bromide. In a few cases the

rubric is not so exacting as in the previous revision. The reasons for such modification are usually self-evident. As examples, ferric chloride is to contain 20 per cent. of Fe instead of 22 per cent., and thymol iodide 43 per cent. of iodine instead of 45 per cent.

A noticeable improvement is seen in the pharmacognostic descriptions. Here we have not only the microscopic appearance and structure of the drug described, but also descriptions of the powdered drug under the microscope. The purity rubric has been extended to the organic drugs, and these monographs commonly give the percentage of allowable admixtures of other parts of the drug plants or other foreign matters. In the organic drugs and their preparations that permit of chemical assaying, limitations are likewise fixed for the variability naturally existing in the drugs and the personal equation or error introduced in the assay processes, and in each case the alkaloidal percentage is fixed by an upper and lower limit.

The assay processes introduce several changes, such as the use of purified saw dust as a distributing medium, and the adoption of the aliquot part method. Cantharides has a definite standard of cantharidin of not less than 0.6 per cent., and an assay process is now given. In nux vomica, the percentage of total alkaloids is fixed at 2.5 per cent. in place of 1.25 per cent. strychnine, and the preparations of nux vomica are all assayed for total alkaloidal content. Opium must now contain not less than 9.5 per cent. *anhydrous* morphine instead of not less than 9 per cent. *crystallized* morphine, and the lime method of assay is adopted. This increase in strength is to be noted, and likewise the determination of morphine content in the anhydrous form instead of that of the crystallized alkaloid.

Assay processes have been extended to numerous preparations not previously assayed. Among such may be mentioned benzoic and salicylic acids, and citrated caffeine; and among preparations, liniment of camphor and spirit of camphor, for which a polariscope method for the determination of camphor is now introduced. Very few pharmaceutical laboratories have the expensive polariscopes required. Hence I fear that this test is more academic than practicable, and its observance in practice will be largely confined to the State Laboratories.

The Ninth Revision will be noted for the number of innovations in pharmacopœial revision. Among these may be mentioned "Electrolytic Determination," which is especially recommended as the method for assaying zinc and mercury compounds; official methods

for the "Determination of Ash," "Saponification Values," "Acid Number of Resins," "Determination of Crude Fibre," "Volatile Extractive and Non-volatile Extractive," "Determination of Alcoholic Content," "Melting Points," "Boiling Points," and "Congealing Points," with a description of "Standard Thermometers, and Solubilities." The chapter on "Sterilization" should be carefully studied by every dispenser. The chapter on "Diagnostical Reagents and Clinical Tests" is an important addition to the U. S. P., in which the example of the later revisions of some of the foreign pharmacopœias, notably the German Pharmacopœia, is followed. This contains standard formulas for the reagents used in the examination of urine, gastric contents, blood, and microörganisms.

For the first time in the history of pharmacopœial revision, the methods for the biological assaying of drug products have been recognized. Chapter 23, in Part 2, is devoted to this subject, and official processes are described by which the following drugs and their preparations *may be* assayed: aconite, digitalis, strophanthus, squills, and dried suprarenals, and cannabis *must be* assayed by the official biological process. The standard adopted for the latter drug is that "Cannabis made into a fluidextract in which one hundred mils represent one hundred grammes of the drug when assayed biologically, produces incoördination when administered to dogs in a dose of not more than 0.03 mil of fluidextract per kilogramme of weight."

The titles added to the list of pharmacopœial substances are only 66 in number. Some of these have been added because of their use as medicines necessitating standards. Among such may be mentioned ethyl morphine hydrochloride, diacetyl morphine and diacetyl morphine hydrochloride, sodium cacodylate, glycerophosphates of calcium and sodium, creosote carbonate, and phenolphthalein.

Commercial conditions necessitated several changes. As examples: virgin scammony not being now obtainable, scammony root was introduced as the source for making the official scammony resin, and sodium cyanide replaces potassium cyanide. The high price of potash salts, due to the war, has been recognized, and permission is given to substitute the sodium carbonates in place of the potassium carbonates in solutions of magnesium citrate, rhubarb preparations, etc.

Several of the additions became necessary because they are ingredients in the formulas of the Pharmacopœia. Among these may be mentioned glucose, directed as a diluent for solid extracts; purified kieselguhr as a filtering medium, oil of sesame as an ingredient in liniments, and sodium indigotindisulphonate, introduced as a coloring for corrosive sublimate tablets, which are to be "tablets of an angular shape (not discoid), having the word "POISON" and the skull-and-crossbones design distinctly stamped upon each, and to contain 0.45 Gm. to 0.55 Gm. corrosive sublimate, and the tablets to be colored blue."

The preparations added have not been numerous, the tendency being to leave to the National Formulary the providing of formulas for preparations. Milk of bismuth and milk of magnesia are two of the popular remedies, however, that have been given pharmacopœial standing.

Pharmacists will be pleased to learn that the list of powdered extracts has been greatly extended, and in several instances formulas for both the pilular extract and the powdered extract of the same drug are very properly given. The instructions of the Pharmacopœial Convention to adopt general formulas wherever possible has been partially carried out by the introduction of general instructions in the aromatic waters, and by general formulas and classifications in the fluidextracts and tinctures.

Two hundred and forty-two titles have been dismissed from the Pharmacopœia. Thirty-eight of these were fluidextracts, seven were pills, and ten were tinctures. A majority of these preparations have been included in the Revised National Formulary, and so the National Formulary will relatively become more important because of these deletions from the Pharmacopœia.

Twenty-nine changes in the Latin titles have been made, and 28 changes in the English names. As examples: alcohol absolutum is now alcohol dehydratum; aqua hydrogenii dioxidi is now liquor hydrogenii dioxidi; cardamomum is now cardamomum semen, the decorticated seed being the official drug; elixir adjuvans is now elixir glycyrrhizæ; hyoscinæ hydrobromidum is now scopolaminæ hydrobromidum; rhamnus purshianæ is now cascara sagrada.

One of the minor changes that has attracted, nevertheless, a great deal of attention is the adoption of the word "millilitre" in place of "cubic centimetre," and the word "mil" in place of "Cc."

In this we have followed the example of the British Pharmacopœia and accepted the authority of the United States Bureau of Standards for the sake of absolute accuracy.

Synonymy will not be treated through the Index as in the Eighth Revision, but following each Latin title will be the official English title and the more commonly used synonyms. In addition to this, there will be the official abbreviation printed in heavy type, with the hope that physicians will adopt these official abbreviations in prescription writing, so that there will be an official authority for the abbreviations for the official titles commonly used in prescriptions.

SOME FALLACIES REGARDING PHENOL.[1]

A Review with Reports of Observations on the Influence of Ethyl Alcohol on the Germicidal and on the Toxic Properties of Phenol.

By Martin I. Wilbert, Technical Assistant, Hygienic Laboratory, United States Public Health Service.

There are probably few official drugs regarding which more misleading statements have been made than phenol, or, as it is more widely known, carbolic acid. This substance was first recognized by Runge (1834), who called it carbolic acid to indicate its nature and origin; an oil-like liquid, obtained from coal, that has much in common with well-known acids. Phenol was early confounded with creosote, isolated by Reichenbach (1832) from beechwood tar, and under the name coal-tar creosote an impure commercial phenol was long listed and freely sold to less well-informed dealers, who unknowingly substituted this more poisonous commercial product for beechwood creosote for internal use.

With the advent of crystalline phenol and its subsequent use as an antiseptic in surgical practice, better informed medical practitioners began to appreciate the difference between the two products, but even at the present time it is not uncommon to find commercial grades of phenol referred to as coal-tar creosote.

The widespread use of phenol as an antiseptic and a disinfectant by medical practitioners served to bring it to the attention of the

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[1] Reprint from the *Public Health Reports,* vol. 31, No. 17, April 28, 1916, pp. 1046–1054.

laity as a poison, and as early as 1890 it was asserted that phenol or carbolic acid was employed more frequently by suicides than any other drug.

The toxicology of carbolic acid early attracted attention, and a record of the substances that have been recommended as antidotes for phenol poisoning, with a review of the reasons for recommending them, would be an interesting study, in that it would tend to emphasize the futility of basing conclusions on incomplete or at times misleading observations.

The use of fixed oils, of glycerin, and of diluted sulphuric acid, and the use of soluble sulphates of the alkalies and alkali earths, while apparently justified on the basis of the earlier observations, have long since been recognized as being inefficient and in many instances distinctly harmful.

The rather widespread use of ethyl alcohol as an antidote for phenol poisoning and the studious avoidance of ethyl alcohol as a diluent for phenol used as an antiseptic or disinfectant, while long since shown to be based on erroneous reasoning, still persist, and, as will be noted later, the belief in the efficiency of ethyl alcohol as a detoxicant for phenol appears to be growing rather than decreasing.

It was early found that alcohol is a better solvent for phenol than is water, and it was also found that mixtures of phenol with alcohol, fixed oils, glycerin, or camphor were less caustic than phenol alone, and under some conditions appeared to be less toxic than solutions of phenol in water.

Glycerin, it was early observed, will lessen the caustic local action of phenol on the skin, but experience has since shown that it will not prevent the production of gangrene nor the absorption of phenol.

A mixture of phenol and glycerin was recommended by Nathan Rosewater and others [2] as a safe and efficient substitute for phenol. In recommending this mixture, it was pointed out that "not being as caustic as phenol, it cannot result in as much mischief or fatality if taken internally, either accidentally or on purpose."

Harrison Allen, as editor of "A Handbook of Local Therapeutics" (Philadelphia, 1897), makes the assertion that "carbolic acid dissolved in oil or in alcohol is inert. Anthrax spores were found to be unaffected after lying upward of three months in a 5 per cent. solution of carbolic acid in oil and equally so by 70 days'

[2] American Journal of Pharmacy, 1895, vol. 67, p. 221.

exposure to a 5 per cent. solution in alcohol. Even the sensitive anthrax bacilli were not destroyed by a 5 per cent. solution of carbolic acid in oil."

Dr. Seneca D. Powell, of New York, was among the first to systematically recommend the use of alcohol as an antidote for phenol. He based his recommendation on the naïve but evidently fallacious deduction that the action of alcohol in the stomach must be analogous to its action on the unbroken skin.

Phelps [3] appears to have been the first to call attention in print to the antagonism of alcohol to phenol. He quotes Dr. Seneca D. Powell, who in his clinics at the Postgraduate Hospital demonstrated the antidotal value of alcohol by consecutively rinsing his hands in liquid phenol and then in alcohol.

Since that time alcohol has frequently been recommended and largely exploited as an antidote to carbolic acid, despite the fact that it is of little value other than as a diluent. The exploitation of alcohol as an antidote and as a possible prophylactic for phenol poisoning has led to its recognition in state and other laws designed to restrict the sale and use of various poisons.

Williams [4] was among the first to make the suggestion that, "in view of the frequently made assertion that grain alcohol is an efficient antidote for carbolic acid and that this poison may be taken with impunity if immediately followed by alcohol, it would appear that a mixture of phenol and grain alcohol would be a comparatively safe household preparation. The claim of the comparative innocuousness of carbolic acid under the conditions named is apparently well founded."

This fallacious suggestion has been embodied in several state and local laws and regulations designed to restrict the sale of carbolic acid. These laws usually provide that the requirements embodied therein do not apply to the sale of crude carbolic acid or to the sale of a solution or mixture containing equal proportions of carbolic acid, glycerin, and alcohol. That this misleading statement, originally made more than twenty years ago, is still a factor in the enactment of restrictive legislation is apparent from a paragraph embodied in the recently (1915) enacted laws of California and of Utah. These laws provide that the restrictions relating to the sale of carbolic acid do not apply to solutions of carbolic acid ("phenol") containing not

[3] *New York Med. Jour.*, 1899, vol. 69, p. 62.

[4] *Drug. Circular*, March, 1900, p. 46

over 10 per cent. of carbolic acid ("phenol") and not less than 10 per cent. of ethyl alcohol.

The same line of reasoning which led to the belief that ethyl alcohol is an efficient prophylactic and antidote for phenol because of its power of removing phenol from the skin also led to a rather widespread belief that mixtures of phenol with alcohol or solutions of phenol and alcohol in water are less efficient as antiseptics or disinfectants.

Taylor,[5] in a report of an experimental study with alcohol-resistant yeasts to determine the antagonism of alcohol to phenol, concludes that this supposition appears to have some physical basis, but is not due to any chemical detoxication of phenol by ethyl alcohol. From his experiments he concludes that alcohol does not reduce in the least the antiseptic action of carbolic acid, the toxicity of the phenol not being at all involved. With a high concentration of alcohol and a low concentration of phenol the alcohol seemed to increase to some extent the antiseptic value of the phenol.

Taylor concludes "that there is no chemical detoxication of phenol by ethyl alcohol and that the effects observed in therapeutic practice must rest upon some physical basis." He also points out that recent investigations by Sollmann support this conclusion.

Zemp[6] appears to have been among the first to question seriously the value of ethyl alcohol as an antidote to phenol. He says: "That alcohol is a splendid solvent for many drugs is recognized by all. It is because of this power that it has been recommended as an antidote to carbolic acid. No chemical action takes place when these two drugs are brought together. The carbolic acid is simply diluted, hence its caustic power is diminished."

Macht[7] reports an experimental study of lavage in acute carbolic acid poisoning in which he clearly demonstrates that, contrary to popular experience and belief, the internal use of alcohol in cases of phenol poisoning may be unfavorable. The conflicting opinions in regard to the use of alcohol are somewhat reconciled by his investigations. He finds that the influence of alcohol depends on the time of administration. If it is given after the ingestion of phenol, as must be the case therapeutically, the symptoms will be aggravated, the alcohol acting as an excellent solvent for phenol, promoting rather

[5] *Jour. Biol. Chem.*, 1908–9, vol. 5, p. 319.
[6] *New York Med. Jour.*, 1909, vol. 89, p. 476.
[7] *Johns Hopkins Hosp. Bull.*, 1915, vol. 26, pp. 98–104.

than retarding its absorption, so that death may actually be hastened. On the other hand, he found that an animal previously intoxicated with alcohol can withstand better the effects of phenol taken afterwards.

To determine the relative influence of ethyl alcohol and of glycerin on the actions of phenol it was thought desirable to repeat in a modified way some of the experiments previously reported. The results of these experiments are appended and clearly show that ethyl alcohol in the presence of water has no appreciable influence on the toxicity or on the germicidal properties of phenol, and that it may therefore be advantageously used as a solvent alone, or in mixtures to promote the solubility of phenol in water for use as a germicide or disinfectant.

The experiments to determine the germicidal value of mixtures of phenol and alcohol and of phenol and glycerin were made in the Hygienic Laboratory by Mr. Albert F. Stevenson and Miss Rose Parrott.

The technic followed was that described in *Hygienic Laboratory Bulletin* No. 82, "The Determination of Phenol Coefficient of Some Commercial Disinfectants," by Thomas B. McClintic.

The results, as evidenced in the appended tables, clearly show that in the presence of water both alcohol and glycerin are practically inert so far as any detoxicating action may be concerned.

In the presence of a larger percentage of alcohol there appears to be some increased activity, due probably to a slight increase in the solvent and penetrative properties of the mixture.

An abstract of a report on the effect of alcohol on the toxicity of phenol, made by Dr. Liston Paine, Assistant Surgeon, United States Public Health Service, is also appended. The results noted serve to emphasize the findings previously reported and suggest the fallacy of enacting legislation designed to promote the sale of mixtures of phenol and alcohol under the impression that ethyl alcohol will serve as a detoxicant to phenol.

In conclusion, it may be again noted that the experimental work clearly shows that the addition of ethyl alcohol to phenol not only increases the solubility of phenol in water, but also increases rather than diminishes the antiseptic value of the resulting solution. Ethyl alcohol can be used to advantage as a substitute for glycerin in making antiseptic solutions of phenol.

The experiments with animals clearly show that the addition of ethyl alcohol to solutions of phenol in water does not, in any way, inhibit the toxic action of phenol, but rather tends to facilitate absorption and thus hasten death.

The Effect of Alcohol or Glycerin on the Toxicity of Phenol as Shown by Inoculations into White Mice.

(An abstract of a report by Dr. Liston Paine, assistant surgeon, United States Public Health Service.)

For the experiments recorded in the appended tables the technic was practically as outlined in *Hygienic Laboratory Bulletin* No. 88, "Method for Determining the Toxicity of Coal-tar Disinfectants," by Worth Hale.

The mice used were prepared in the afternoon of the day before they were to be injected, so as to insure a maximum of time for observing the development of symptoms.

The symptoms manifested by the mice referred to in the accompanying tables were typical of phenol poisoning. The mice that were injected with a mixture of phenol and alcohol or phenol and glycerin showed the symptoms just as early and to as marked an extent as the mice receiving phenol alone.

To determine the effect of ethyl alcohol, a solution containing twice as much alcohol as the maximum amount used with phenol was injected. All of these mice recovered within 24 hours, though all were stupefied from the effect of the alcohol and three appeared to be moribund.

On injecting the same mice on the following day with an aqueous solution to determine whether such previously alcoholized mice could better resist the toxic action of phenol it was found that three died from approximately the same dose that proved fatal for other animals. (See Table E.) It should be noted in this connection that in this series of mice the phenol was injected after the mice had apparently recovered from the effects of the alcohol. It is probable that most of the alcohol had been excreted through one or another channel within the intervening 24-hour period.

In the appended tables an effort has been made to include only the essential information recorded in the protocols. The dose per mouse and dose per gramme of mouse represent the weight of phenol in the solutions used.

TABLE 1.

Standard Phenol in Water.

RESULTS OF A TEST (WITHOUT ORGANIC MATTER).

(+ means growth; – means no growth.)

Sample	Dilution	Time culture exposed to action of disinfectant in minutes					
		2½	5	7½	10	12½	15
Phenol	1:80	–	–	–	–	–	–
	1:90	–	–	–	–	–	–
	1:100	+	–	–	–	–	–
	1:110	+	+	–	–	–	–
	1:120	+	+	+	+	+	–

TABLE 2.

A Mixture of Phenol 1 and Glycerin 1 in Water.

RESULTS OF A TEST (WITHOUT ORGANIC MATTER).

(+ means growth; – means no growth.)

Sample	Dilution	Time culture exposed to action of disinfectant in minutes					
		2½	5	7½	10	12½	15
Phenol	1:80	–	–	–	–	–	–
	1:90	–	–	–	–	–	–
	1:100	+	–	–	–	–	–
	1:110	+	+	–	–	–	–
	1:120	+	+	+	+	+	–

TABLE 3.

A Mixture of Phenol 1 and Alcohol 1 in Water.

RESULTS OF A TEST (WITHOUT ORGANIC MATTER).

(+ means growth; – means no growth.)

Sample	Dilution	Time culture exposed to action of disinfectant in minutes					
		2½	5	7½	10	12½	15
Phenol	1:80	–	–	–	–	–	–
	1:90	–	–	–	–	–	–
	1:100	+	–	–	–	–	–
	1:110	+	+	–	–	–	–
	1:120	+	+	+	+	+	+

TABLE 4.

A Mixture of Phenol 1 and Alcohol 3 in Water.

RESULTS OF A TEST (WITHOUT ORGANIC MATTER).

(+ means growth; – means no growth.)

Sample	Dilution	Time culture exposed to action of disinfectant in minutes					
		2½	5	7½	10	12½	15
Phenol....................	1:80	–	–	–	–	–	–
	1:90	–	–	–	–	–	–
	1:100	+	–	–	–	–	–
	1:110	+	–	–	–	–	–
	1:120	+	+	+	–	–	–

(To be continued)

BOOK REVIEWS.

THE PHARMACOPŒIA OF THE UNITED STATES OF AMERICA. NINTH DECENNIAL REVISION. By authority of the United States Pharmacopœial Convention held at Washington, D. C., May 10, 1910. Prepared by the Committee of Revision and published by the Board of Trustees. Official from September 1, 1916. Philadelphia Agents: P. Blakiston's Son & Company. Sub-agents: New York, Paul B. Hoeber, 67 East Fifty-ninth Street; Chicago, Chicago Medical Book Company, Congress and Honore Streets; St. Louis, Lewis S. Matthews & Co., 3563 Olive Street; San Francisco, H. S. Crocker Company, 565 Market Street.

The Ninth Revision of the United States Pharmacopœia is out at last. While the delay in getting out this revision may have been disconcerting to those desiring the new revision, yet this is more than compensated by the excellence of the new work. Probably in connection with no revision have so many new features been introduced as in the Ninth Revision, and this required the revisers to carefully consider all the questions involved and to render a final decision only after mature judgment could be formed. It is not our object in this review to go over in detail this new book, as we publish elsewhere in this issue an excellent summary of the main features of this revision. A few facts from the Preface and Introductory Notices may be sufficient to indicate some of the changes that have been adopted. The scope of the Ninth Revision is indicated by the following: "The consensus of opinion of the committee was, in effect, to provide standards for vegetable drugs, chemical substances, and such pharmaceutical preparations as were simple in their character, and

most largely used. A few compound preparations, however, were retained because of their large use; an increase has been made in the number of standardized serums and animal products. A number of synthetic remedies have been added to the list which the Subcommittee on Scope had recommended for admission, permission having been granted by the manufacturer, firm, or corporation to include such substances; unfortunately the European war has interfered with the receipt of some answers from foreign countries. In most cases where answers were received the replies were in the negative."

It is stated that "The class of preparations known as 'Wines' has not been included in this revision. Wine as a menstruum or solvent can with advantage be replaced by alcohol of various strengths, and the uncertainties, due to the variability in quality and alcoholic content of the wines of commerce, are avoided."

In regard to number of admissions and deletions, "The number of articles, reagents, and assays in the present Pharmacopœia is 1436; there were 1297 in the previous Pharmacopœia. In the present book there are 782 articles in the text; 277 test solutions and volumetric solutions, 315 volumetric, gravimetric, and other assays, and 62 diagnostical reagents. In the U. S. P. VIII there were 958 articles in the text, 155 test solutions and volumetric solutions, 149 volumetric assays, and 35 gravimetric assays. Of those articles official in the text of the U. S. P. VIII, 243 have been dismissed, while 67 new ones have been introduced into the U. S. P. IX."

One of the most interesting changes is the replacement of Cc. by Mils. "The United States Bureau of Standards declared that the term cubic centimetre was a misnomer, there being a slight difference between the thousandth part of a litre and the cubic centimetre, as one litre was determined to be equivalent to 1.000027 cubic decimetres. The Committee of Revision decided that the time had come to adopt the word mil, the first three letters of the whole word millilitre. In addition, the change promotes international uniformity in the two Pharmacopœias published in the English language."

The language of the chemical tests has been changed, the imperative mood being used in place of the conditional form of previous Pharmacopœias. In former revisions nearly every test began, "If — Gm. be dissolved." In the present revision it is changed to the imperative "Dissolve — Gm., etc." The Appendix is now designated as Part II, Table of Contents precedes it, and a more systematic arrangement has been adopted. "Much revision was found

necessary in this part of the work, and the list of reagents has been enlarged. While a knowledge of the chemical analysis is presupposed, the committee has deemed it not out of place to add explanatory remarks or instruction in the details of manipulations in Part II." Furthermore, "In the chemical tests an extension has been made of the plan employed in the previous Pharmacopœia where general tests were adopted for arsenic and heavy metals. The additions will be found in Part II. The adoption of this plan saves space by avoiding frequent repetition of detail in the text of Part I."

Probably the greatest departure in the IX U. S. Pharmacopœia is in the text for vegetable drugs. For the first time the histological treatment has been introduced throughout the work. The microscopic structure of the crude drug has been introduced wherever necessary, and the microscopical characteristics of powders are in nearly all cases given. . It is stated in the Introductory Notice: "In the case of vegetable drugs, the standards provided in the text apply also to the powdered or ground drug. For the preservation of vegetable or animal substances from the ravages of insects, it is directed in special cases that they be preserved in tightly-closed containers and a few drops of chloroform or carbon tetrachloride added. It is not intended that this precaution should be used for drugs imported in bales or large original containers. This precaution is intended to aid in the preservation of drugs in the stock of a pharmacist."

The official date on which the IX U. S. Pharmacopœia goes into effect is September 1, 1916.

SQUIRE'S COMPANION TO THE BRITISH PHARMACOPŒIA (1914). Nineteenth Edition, demi-octavo, pages 1712. J. and A. Churchill, 7 Great Marlborough Street, London, W. Price, 15/-net, post-free (inland) 15/7d.

The new Nineteenth Edition of Squire's Companion to the British Pharmacopœia contains a systematic and critical record of the alterations and additions to the new British Pharmacopœia, showing which preparations are new, which are stronger, or which are weaker, the alterations being plainly indicated in the text by the adoption of distinctive markings; *e.g., New, Altered,* or *Modified,* as the case may be. The British Pharmacopœia is also critically compared with the United States and German Pharmacopœias, this

criticism being extended in important instances to a comparison with the French and Swiss Pharmacopœias.

The arrangement of matter, which has been distinctive of Squire's Companion for over fifty years, is continued in the present edition, as this method of arrangement has been found to be the most convenient for ready reference. The Latin and English titles of the substance appear at the head of each monograph, followed by the French, German, and Italian names under which it is known, a short description, together with, in the majority of instances, a brief account of its usual method of preparation, Solubility, Medicinal Properties, Dose, Prescribing Notes, Incompatibles, Official Preparations, Not Official Preparations, Antidotes, Foreign Pharmacopœias and Tests. In the case of the Materia Medica, these paragraphs are supplemented by Descriptive Notes, which have been revised and brought up to date by Mr. E. Morell Holmes.

The paragraphs on Solubility introduce a number of figures specially determined for this edition, while in those cases in which there has been reason to doubt the accuracy of figures appearing in the previous editions these have been either confirmed and the old figures retained, or, where necessary, the figures have been modified to meet the new conditions. The paragraphs on Solubility have formed a distinctive feature of Squire's Companion since 1864, and is one of the most valuable practical features of the book.

The paragraphs on Medicinal Properties have been entirely revised and brought thoroughly up to date, some of the references being as late as April, 1916. They form an exhaustive review of over half a century's progress in therapeutics. In order to make this review as complete and useful as possible, the more important of the older and obsolete matter has been condensed and incorporated in the general text of the book, while the unimportant part has been entirely deleted. The uses of hypochlorous acid as an antiseptic are included under Calx Chlorinata, on page 367. With descriptions of Eupad and Eusol, the new antiseptic Chloramine is referred to in the supplementary matter on page 1541. An account of the Iodine treatment of wounds appears on page 771. The latest compounds used in syphilis are referred to on page 1189 to 1198, and include Kharsivan, Neo-Kharsivan, Galyl, Hectine, and Intramine.

The Prescribing Notes represent the author's personal experience of over forty years in the dispensing of physicians' prescriptions. In many instances the information regarding the dispensing of a

particular drug is embodied in the form of a Prescribing Note in preference to introducing it under the heading of Not Official Preparations. Typical instances appear under Acetanilide, page 8; Acidum Boricum, page 33; Acidum Carbolicum, page 40; Ergot, page 568, and Tinctures, page 1400.

Doses are given in the Imperial and Metric systems. In the case of potent remedies the pharmacopœial dose is compared with the maximum single and maximum daily dose of one or more of the important continental pharmacopœias.

A list of Official and Not Official Preparations is given under the heading of each important substance. The paragraphs on Foreign Pharmacopœias compare the substance under review with 17 other foreign pharmacopœias.

A reference to the Preface shows that the paragraphs on Tests have been entirely rewritten; these paragraphs not only furnish a critical comparison of the tests appearing in the British with those appearing in the United States and German Pharmacopœias, but this is supplemented, in important instances, by a comparison of the tests given in the French and Swiss Pharmacopœias. Very many references are made to the work done in the laboratory of the author. They comment on and supplement the Official Tests, and in some instances show that tests which are now Official in the British Pharmacopœia have been described in previous editions of Squire's Companion. A great deal of attention is devoted to the standardized preparations of the British Pharmacopœia, which are very thoroughly compared with those of the other continental pharmacopœias.

In dealing with the special chapter on Chemicals, Reagents, etc., used in qualitative testing, volumetric analysis, indicators, and special tests, the same comparative method has been adopted, the British being compared with the French and German Pharmacopœias. Methods are given for the determination of physical constants and analytical memoranda relating to special tests, and include such constants as melting-points, solidifying points, boiling-points, optical rotation, refractive index, specific gravity, arsenic and lead tests, acid and saponification values, unsaponifiable matter, and the determination of esters and alcohols in volatile oils.

The concluding sections of the book are occupied by a description of Spas, enumerating first those of Britain and subsequently the foreign Spas, a classification of mineral waters and a therapeutical classification of remedies, and a general index. In order to complete

the utility of the book as a work of reference, the index has been printed in specially bold type, and a clear differentiation has been made between substances which are Official and those which are Not Official, the former being printed in Roman and the latter in italic type.

PENNSYLVANIA PHARMACEUTICAL ASSOCIATION.

With the largest registration in its history, the thirty-ninth annual meeting of the Pennsylvania Pharmaceutical Association at Reading, Pa., on June 20, 21, and 22, was not only the largest and most successful of any of its predecessors, but demonstrated most conclusively that the centrally located city as a meeting place can be made just as attractive—possibly more so—as the mountain resort. Not since 1899 has the Pennsylvania organization held its sessions at other than a summer resort, and the innovation, it was feared, would not be a success. But the contrary was proved most emphatically.

Discussion and action upon legislative issues dominated the business sessions of the association. An itinerant venders' bill in revised form, it was decided, should be presented by the incoming Legislative Committee at the session, next January, of the Pennsylvania Legislature. The report of Chairman S. C. Henry, of the Legislative Committee, contained a recommendation that the Pennsylvania Drugs Act be changed so as to harmonize entirely with the Ninth Revision of the United States Pharmacopœia and the National Formulary, as well as the Shirley Act. This was approved by the association, as well as the proposed amendments to the Pharmacy Act which will provide for the recognition and registration of those having experience in hospital pharmacies and who are otherwise qualified and can pass the State Board, and will compel hospitals where prescriptions are compounded to have a registered pharmacist in charge. The incoming committee will also endeavor to frame legislation pertaining to the sale and possession of narcotic drugs that will provide for the contingency created by the recent decision of the Federal Courts under which it is no longer considered a misdemeanor for persons other than those properly registered to have the restricted narcotic drugs in their possession.

The Stevens-Ashurst Bill was endorsed; affiliation with the National Association of Retail Druggists continued, and honorary

membership, upon the recommendation of retiring President Theodore Campbell, conferred upon W. B. Day, of Chicago, Ill., general secretary of the American Pharmaceutical Association.

The election of officers resulted as follows: President, Adolph Schmidt, McKeesport; first vice-president, M. W. Bamford, Reading; second vice-president, W. H. Knoepfel, Scranton; secretary, R. P. Fischelis, Philadelphia; assistant secretary, L. H. Davis, Philadelphia; local secretary, B. W. Pritchard, Pittsburgh; treasurer, P. H. Gliem, Lebanon; member of the Executive Committee, D. J. Reese, Philadelphia. Mr. Reese had been the efficient secretary of the association since 1914, but press of other business made it impossible for him to accept a renomination this year.

The invitation of the Western Pennsylvania delegation to visit them next year was accepted, and the Hotel Schenley, Pittsburgh, was chosen as the meeting place, June 19, 20, and 21, 1917.

The program of entertainment contributed greatly to the success of the meeting. One day, the visitors were the guests of the Reading Chamber of Commerce and the retail druggists of that city in a trolley tour and luncheon at the Tower, a lofty mountain resort, overlooking the city. Wednesday evening, William H. Luden, the well-known confectionery manufacturer of Reading, was host to the convention at a banquet at the Hotel Berkshire, where the sessions were held, while throughout the meeting there were all sorts of scientific and guessing contests, dances, automobile trips, athletic sports, and card parties, designed either for the entertainment of the ladies or for the men when sessions were not scheduled.

The handsome gold prize for the best paper presented at the 1915 session was awarded to Charles H. LaWall, of Philadelphia, while throughout the meeting the association maintained its enviable reputation for the number and excellence of the papers read.

Reading druggists and their good wives proved ideal hosts, while the excellent roads, numerous trolley and steam lines, made it very accessible for druggists from all over the State. Automobile parties from Philadelphia, 58 miles away, were very numerous. That city, as usual, sent the largest contingent.

The report of Secretary Reese, of the parent organization, showed that of the 162 members elected during the year, 135 applications had been secured directly by the Travelling Men's Auxiliary. The salesmen gave an excellent entertainment after the Luden banquet, and proved, as usual, a powerful working arm of the association.

The Auxiliary elected the following officers: President, John Q. Reinhart; vice-president, W. M. Skivington, Philadelphia; treasurer, J. D. McFerren, Philadelphia; secretary, J. W. Garlick, Philadelphia.

PHILADELPHIA COLLEGE OF PHARMACY.

Minutes of the Quarterly Meeting.

The quarterly meeting of the Philadelphia College of Pharmacy was held June 26, 1916, at 4:15 P.M., in the Library, the President, Howard B. French, presiding. Seventeen members were present.

The minutes of the annual meeting, held March 27, were read and approved. The minutes of the Board of Trustees for March, April, and May were read by the Registrar, J. S. Beetem, and approved.

The report of the Committee on Necrology was read by Prof. Henry Kraemer, Chairman, and is as follows:

During the past year we have lost through death but one member, viz., Dr. Joseph A. Heintzelman. He conducted one of the oldest drug stores in Philadelphia, and died October 19, 1915. Doctor Heintzelman was born in Germany, in 1834. At the age of twenty-four he came to this country, devoting himself for several years to the study of pharmacy and medicine, graduating from the Philadelphia College of Pharmacy in 1859. In 1874 he opened a drug store at the corner of Ridge and North College Avenues, which was then considered a suburban point of Philadelphia. (For a few years prior to this he was located at Tenth and Ogden Streets.) He not only conducted a drug store in this locality, but was a practising physician and very highly esteemed in this community. He played a very important part in the development of this section of the city, and was quite active in a number of German societies and fraternal orders in Philadelphia. Doctor Heintzelman was ill but a short time, and died at the home of his son, Joseph A. Heintzelman, Jr., who graduated from the Philadelphia College of Pharmacy in 1898, and into whose hands the control of the store passed several years ago. He joined the college in 1859, the same year that some of our most distinguished pharmacists, who are now deceased, became members, viz., Prof. John M. Maisch and Robert England.

The report of the Committee on Membership was read by Prof. Charles H. LaWall, chairman. He said: "The committee has been considering revising the roll of membership. A number of them are in arrears and are liable to forfeit their membership. A plan was proposed to meet this condition, which the Secretary was directed to carry out."

The report of the delegates to the New Jersey Pharmaceutical Association was given by Professor Remington, chairman. He said the Association met at Long Branch. There was a good attendance; the meeting was full of life; they were an active and energetic set of men in the Association; it was the most successful meeting for years. The Association is also in good financial condition, and the entertainment which was provided was excellent.

The delegates to the Delaware Pharmaceutical Association reported through Dr. A. W. Miller, chairman. In his absence the report was read by the Secretary:

"The meeting was held on June 1, at the Hotel DuPont, in Wilmington, Del. The fraternal greetings and cordial good wishes of the college were presented, and the members were urged to send their students. The meeting was a small one, perhaps not more than twenty-five or thirty members being present, although at the subsequent luncheon there were probably double this number, on account of the ladies coming in. Only one business session was held, lasting about 2½ hours, after which we were invited to an inspection of the new City Hall and Court House."

The delegates to the Pennsylvania Pharmaceutical Association presented a verbal report through Prof. F. P. Stroup: "The meeting held at Reading was the largest held in recent years, thus proving the wisdom of meeting in the cities or larger towns rather than at summer resorts. Five members of the faculty of the college and a large number of its graduates were among those in attendance. The papers presented were interesting, as in former years. The weather was favorable, the entertainments were very good, and the hotel accommodations excellent. One of the very good effects of the meeting was the influence on the Reading druggists, they becoming consolidated and better known to each other than ever before. The Association meets in Pittsburgh next year."

The proposed amendment to Article VIII, Section 1, of the By-laws, offered at the annual meeting and laid over, was then considered and adopted, as follows: "Article VIII, Section 1.—Any person approving the objects of the college and its code of ethics may be elected an active member."

Dr. P. S. Stout, for the Committee on Centenary Celebration, stated that up to this time $8000 had been subscribed to the fund and about 20 per cent. paid in.

President French read a report of a recent meeting of the committees on the Fiftieth Anniversary Alumni Celebration (see p. 336 of this JOURNAL).

The discussion that followed the reading of the report was interesting and took a wide range. The discussion was participated in by Messrs. Beringer, French, Kraemer, Stroup, and Remington. As a result of the discussion Mr. Beringer moved that a committee of the college be appointed to coöperate or act in conjunction with committees now at work from the New York Pharmaceutical Association, the New Jersey Pharmaceutical Association, and the American Pharmaceutical Association in providing entertainments at the coming meeting of the A. Ph. A. It was so ordered, and the President appointed Prof. Joseph P. Remington, George M. Beringer, Henry Kraemer, Dr. Mitchell Bernstein, and Dr. P. S. Stout.

Dr. Stout alluded to the various prizes given by the college, and suggested that a list of such prizes should be posted in the corridor, as it would prove an incentive to more diligent application on the part of students in their studies. The suggestion was favorably commented on and, on motion, agreed to.

The President appointed the following committees:

On Nominations: Joseph W. England, Warren H. Poley, F. P. Stroup, Dr. Mitchell Bernstein, and Dr. P. S. Stout.

Committee on Necrology: Henry Kraemer, Joseph W. England, and C. A. Weidemann.

Delegates to the meeting of the American Pharmaceutical Association to be held at Atlantic City, N. J., September 5–9, 1916: Prof. Henry Kraemer, Prof. Frank X. Moerk, Prof. Charles H. LaWall, Prof. E. Fullerton Cook, Prof. Freeman P. Stroup, with power to fill vacancies.

C. A. WEIDEMANN, M.D.,
Recording Secretary.

ABSTRACTS FROM THE MINUTES OF THE BOARD OF TRUSTEES.

March 7, 1916.—Seventeen members were present.

The Committee on Instruction reported at length relative to changes in the course of instruction, and proposed a number of recommendations, as follows:

That theses be required from students desiring to graduate with the degree of Phar.D.

That the position of Professor Emeritus of Chemistry be created, and that Prof. Samuel P. Sadtler be elected. Professor Sadtler expressed his appreciation of the honor bestowed upon him.

Both of the above recommendations were adopted.

Mr. Mulford spoke of some of the advantages of student government, and moved that the Committee on Discipline and the Committee on Instruction consider the matter. It was so ordered.

Mr. French stated that the College had been invited to participate in the Philadelphia "To-day and To-morrow" Civic Exposition, to be held in the Commercial Museum, May 15 to June 10, 1916. The subject was referred to the Committee on Announcement to investigate and report.

The Chairman referred to the appropriation asked of Congress for the pedestal for the Procter Monument. The Dean explained the present situation, and suggested that a committee be appointed, representing the College, to coöperate with the National Committee. Being so ordered, the Chair appointed Messrs. Howard B. French, Joseph W. England, Edwin M. Boring, Henry K. Mulford, and O. W. Osterlund.

April 4, 1916.—Eighteen members were present.

A communication was received from the Secretary of the College, reporting the election of officers at the annual meeting of the College, held March 27, 1916 (see pp. 232 and 233 this JOURNAL).

The election of chairman and vice-chairman of the Board of Trustees and registrar for the ensuing year being in order, the following were elected: George M. Beringer, chairman; Walter A. Rumsey, vice-chairman; Jacob S. Beetem, registrar.

Committee on Finance reported the desire of the Treasurer to be bonded, and also moved that this be done at the expense of the College, and, further, that the matter be referred to the Committee on Finance. It was so ordered.

Committee on Instruction reported further relative to the new course in the standardization of drugs. Several titles had been proposed for this course, but none as yet had been selected.

A report was also made relative to the course of instruction to be given students for the Phar.D. degree, and also to the filling of the position of Professor in Chemistry.

The committee recommended the election of Dr. Paul S. Pittenger as instructor in the course of Standardization of Drugs. On motion it was so ordered.

Committee on Examinations reported the name of Chester L. Masser as having successfully passed his examination and that he was, therefore, entitled to receive the Certificate of Proficiency in Chemistry. On motion the certificate was duly awarded.

Committee on Announcement reported that the appointment of a publicity agent had been considered. The committee further reported in favor of making an exhibition at the Philadelphia "To-day and To-morrow" Civic Exposition. The recommendation was approved and an appropriation was made to cover the necessary expenses.

The Chairman announced the standing committees for the ensuing year. The following were named chairmen of the various Committees:

Howard B. French, Property Committee.
Samuel P. Sadtler, Library Committee.
O. W. Osterlund, Museum and Herbarium Committee.
Howard B. French, Finance Committee.
H. K. Mulford, Supplies Comimttee.
George M. Beringer, Instruction Committee.
C. A. Weidemann, Accounts and Audit Committee.
Joseph P. Remington, Scholarship Committee.
William L. Cliffe, Examination Committee.
Joseph W. England, Theses Committee.
Howard B. French, Discipline Committee.
Joseph W. England, Announcement Committee.
Walter A. Rumsey, Commencement Committee.
Joseph W. England, Alumni Committee.

Appropriation Committee, the chairmen of all committees empowered to make expenditures, the chairman of the Board, the chairman of the Committee on Finance, and the Treasurer.

April 17, 1916.—Adjourned meeting. Fifteen members were present, and Prof. Frank X. Moerk, although not a member of the Board, was also present.

Committee on Instruction.—Supplemental report. The various recommendations were acted upon seriatim, as follows:

Recommended that the title of the course in Standardization of Drugs be called "Biologic Assaying." This was adopted.

The recommendation relative to the Phar.D. course and B.Sc. course was approved and referred to the Committee on By-Laws.

The change in the award of the Kappa Psi prize was approved.

The new prize in Dispensing Pharmacy, offered by Prof. E. F. Cook, was accepted and approved.

The changes in the various prizes made necessary by the Ph.G. course were approved.

Mr. C. J. Zufall was appointed Instructor in Botany and Pharmacognosy for the ensuing year. His services are to begin June 1, 1916.

The resignation of Mr. W. F. Haase as student assistant was, on motion, accepted.

Committee on Announcement reported relative to the selection of a publicity agent, and recommended the election of Mr. J. R. Graham for the ensuing year, which was approved.

Mr. French announced that he had secured from the Commercial Museum 15,000 botanical specimens. The thanks of the Board were extended to Dr. W. P. Wilson, director of the Commercial Museum, for the gift.

May 2, 1916.—Eighteen members were present.

Committee on Instruction has continued its meetings and has mapped out the curriculum for the post-graduate courses, leading up to the degree of Doctor in Pharmacy (Phar.D.) and Bachelor of Science in Chemistry and Pharmacy (B.Sc.). The outline for these courses has been practically completed for the Announcement.

The committee recommended that the fee for the Bachelor of Science degree be made $150 per year for each of the four years' instruction. This was approved.

The committee also recommended the establishment of a course in pharmaceutical arithmetic as a department of instruction in the College, and further recommended that Mr. Ivor Griffiths, P.D., of 1912, be engaged to give this instruction. This was approved.

Committee on Examination presented a report from Prof. John A. Roddy, Professor of Bacteriology, giving the names of those students who had satisfactorily completed the special course in Bacteriology. On motion, it was ordered that the Certificates be awarded (see p. 333 of this Journal).

Committee on By-Laws offered an amendment to Article VIII, Section 4, which, according to the rule, was laid over for one month.

Professor Sadtler asked for a consideration of the election of his successor, when, after a general discussion of this important matter, Prof. Freeman P. Stroup was appointed Acting Professor of Chemistry for the session 1916–'17.

Mr. Beringer moved that Professor Stroup select an assistant for the Department of Chemistry, subject to the approval of the Board. It was so ordered.

May 31, 1916.—Special meeting. Fourteen members were present. Regrets were received from four members of the Board.

Committee on Examination presented the names of those who had successfully passed the examinations and were entitled to receive the degree of Doctor in Pharmacy (P.D.). A vote was taken and Mr. Poley, who acted as teller, reported the ballot clear. The Chairman then declared those named elected to receive the degree of Doctor in Pharmacy (P.D.) (see July issue this JOURNAL).

The committee then read the list of candidates for the degree of Pharmaceutical Chemist (P.C.). A vote was taken and, the ballot being clear, the Chair declared them elected to receive the degree of Pharmaceutical Chemist (P.C.). (See p. 333, this JOURNAL.)

Six pharmaceutical chemists of former years having applied for the degree of Doctor in Pharmacy, and complying with the requirements of the College, were voted for and, the ballot being clear, were declared elected to receive the degree.

The names of those entitled to receive prizes were then announced.

The Dean announced that Col. H. A. Demming offered a prize—a ruby, valued at $50—to be given to the student making the greatest improvement over the previous year. The awarding of this prize was to be left to the Committee on Examination.

The Dean also stated that Colonel Demming decided to establish a prize of $20 in gold for the best thesis on Mineralogy. The offer was accepted and the thanks of the Board tendered the donor.

The names of those appointed to present the prizes at the coming Commencement were then announced.

The committee further announced the names of Herbert Calvin Brightbill and Robert Rowen as having taken the full course in Analytical Chemistry, and, passing their examinations, were entitled to receive the Certificate of Proficiency in Chemistry. On motion the certificates were awarded.

Mr. French read a communication from Professor Stroup relative to the High School scholarships. After some discussion, it was voted that an invitation be sent, through the Board of Education, to each of the High Schools in Philadelphia, and the matter be left in the hands of the President.